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Salicylate pharmacokinetics in patients with rheumatoid arthritis

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1 The pharmacokinetics of salicyclic acid (SA) and its major metabolite salicyluric acid (SU) were studied in nine patients with rheumatoid arthritis following a 900 mg oral dose of acetylsalicylic acid and during 6 weeks of chronic administration of enteric coated aspirin (3,900 mg day). Response to therapy was also monitored.

2 The various pharmacokinetic parameters determined in the study were similar to those observed in other single dose salicylate studies amongst healthy volunteers but were not predictive of salicylate concentration in the chronic dose study.

3 Plasma concentrations of SA (total and unbound) were found to decline significantly over the 6 weeks and plasma SU concentrations increased.

4 During the chronic dosing study, there was a significant increase in the V_{max} (total and unbound) for the formation of SU, whilst the K_m and SU clearance remained constant. Also, the elimination rate constant (k) for salicylate was not significantly affected.

5 Therapeutic response to salicylate therapy was not significantly affected by the decline in SA concentrations.

Keywords salicylic acid salicyluric acid rheumatoid arthritis single-dose study chronic treatment pharmacokinetics Michaelis Menten constants

Introduction

Salicylates have been used in the treatment of rheumatoid arthritis (RA) for almost 100 years, although the anti-inflammatory effect of salicylate has only been recognised in relatively recent times (Boardman & Hart, 1967). Rheumatoid arthritics are perhaps the largest group of patients receiving long term aspirin/salicylate therapy. In spite of this, comparatively few pharmacokinetic studies of salicylate have been conducted in this group of patients. The pharmacokinetics of salicylate have been studied extensively in healthy volunteers and have been reported to be complex; both protein binding (Borgå *et al.*, 1976) and elimination kinetics (Levy & Tsuchiya, 1972) being saturable.

Plasma salicylate concentrations above 150 μg/mL are said to be necessary to obtain a significant anti-inflammatory response (Koch-Weser, 1972) and, although oral aspirin doses greater than 3900 mg/day will generally result in an adequate concentration, genetic factors (Furst *et al.*, 1977) and the complexity of the pharmacokinetics often make it difficult to achieve this therapeutic plasma concentration. This seems particularly true of patients with rheumatoid arthritis (Graham *et al.*, 1977; Günsberg *et al.*, 1984).

Several methods of predicting the steady-state drug concentration in plasma have been proposed. One such method (Graham *et al.*, 1977) suggested that pharmacokinetic parameters determined from a single-dose of soluble aspirin, sufficient to saturate the capacity-limited pathways, may be useful in predicting steady-state plasma drug concentrations. The original paper demonstrated the efficacy of the method among healthy volunteers and RA patients.

In addition to the problem of variable steady-

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state drug concentrations, studies in healthy subjects have reported a significant decline in steady-state plasma salicylate concentration during chronic administration of therapeutic doses of aspirin (Müller *et al*, 1975; Olsson 1983; Rumble *et al.*, 1980). Many questions about the actual nature of this observed decline and associated efficacy also remain unanswered.

Rumble *et al.* (1980) reported that the decrease could not be attributed to factors associated with the absorption of the drug or changes in renal function. In the absence of other explanations, the decrease in plasma salicylate concentrations has been attributed to induction of the formation of salicyluric acid. Furst *et al.* (1977) observed a significant increase in the V_{max} for salicylurate formation from 0.92 mg kg^{-1} h^{-1} to 1.36 mg kg^{-1} h^{-1}, after only 3 days of oral aspirin therapy in 26 healthy subjects, while Rumble *et al.* (1980) observed a nonsignificant increase in the urinary output of salicylurate during chronic dosing. Day *et al.* (1983, 1988) suggested that the induction process was time-limited, with no further increase in V_{max} after 2 weeks. A time and perhaps dose related increase in V_{max} during chronic therapy was also described by Günsberg *et al.* (1984). The time course of the decline in steady-state salicylate concentrations appears to be subject to greater variations; in some individuals it occurs rapidly and is limited, whilst in others it appears to be gradual and continuous (Olsson, 1983). There has been no examination of salicylurate plasma concentrations during chronic aspirin therapy. In the study by Day *et al.* (1983) aspirin was stopped and washed out prior to the determination of V_{max} using urine data, while in the study by Günsberg and associates (1984), plasma salicylate data were used to calculate V_{max}.

The importance of the observed decline in salicylate concentrations to therapeutic efficacy is unknown. Although reference has been made to the development of tolerance to aspirin during chronic therapy in patients with RA (Rumble *et al.*, 1980), no controlled study of the phenomenon has been performed. Whether or not a plasma drug concentration-response relationship exists in salicylate therapy is open to debate. Günsberg *et al.* (1984) reported a concentration-response relation, however other authors (e.g. Ekstrand *et al.*, 1979) have been unable to demonstrate any significant relationship between dose or concentration and response. Nevertheless it is widely reported in the literature that a plasma salicylate concentration greater than 150 μg ml^{-1} is required for anti-inflammatory effects. All studies observing a decrease in steady-state salicylate concentration during chronic aspirin administration to healthy volunteers (Müller *et al.*, 1975; Olsson, 1983; Rumble *et al.*, 1980), reported subjects in whom the plasma drug concentrations had declined to below 150 μg ml^{-1}. No similar studies have been conducted in patients with rheumatoid arthritis.

The aims of the current study were to determine whether or not there is a decline in both total and unbound salicylate concentration during chronic aspirin therapy in patients with rheumatoid arthritis; then to define the pharmacokinetics of salicylate in these patients during chronic dosing, using both salicylic and salicyluric acid, unbound and total plasma concentrations, together with urine data. Initial pharmacokinetic parameters were determined for each subject in a 900 mg single dose salicylate study. These parameters were also evaluated for their value in predicting steady state salicylate concentration during chronic therapy. An attempt was also made to assess the significance of any decline in steady state salicylate concentrations.

Methods

Subjects

Patients with classical or definite rheumatoid arthritis, according to the ARA criteria (Ropes *et al.*, 1958), were considered for the study. Patients taking slow acting antirheumatic drugs (SAARDs) and/or corticosteroids were required to have been stable on this therapy for six or more months. Patients with peptic ulcers or any other serious medical problems were excluded. The protocol for the study was approved by the Faculty of Medicine Human Ethics Committee and subsequent to giving written informed consent, nine patients were entered into the study. Their mean age (± s.d.) was 56.7 (± 7.5) years and the average duration of disease was 14.6 ± 14.5 years.

Study design

All previous aspirin therapy was ceased at least 2 weeks prior to the start of the single dose study. Subjects who had been on regular aspirin therapy prior to this were maintained on diclofenac and paracetamol. After a single day washout of all non-steroidal anti-inflammatory drugs (NSAID) and an overnight fast, the subjects were admitted to a hospital day ward. All subjects were weighed and given a single dose of 900 mg aspirin (3 × 300 mg soluble aspirin) dissolved in 100 ml of water. Immediately before the dose

(0 min) 10 ml of venous blood were drawn from an indwelling cannula for measurement of baseline drug concentrations, ESR and to perform full serum biochemistry and liver function tests. Subsequently 5 ml blood samples were collected at approximately 15, 30, 45, 60 min and 2, 3, 4, 6, 8, 10 and 24 h after the dose. In order to minimise hydrolysis of aspirin, all blood samples taken in the first 4 h were collected into chilled centrifuge tubes containing 2.5 mg of potassium fluoride. After separation these samples were stored at −20°C and assayed by high-performance liquid chromatography within 72 h for acetylsalicylic and, salicylate and salicylurate using the assay of Rumble *et al.* (1981). Unbound plasma concentrations were measured using ultrafiltration and h.p.l.c. analysis.

All urine was collected for 24 h after the dose. Total urine salicylate was measured using the method of Rumble *et al.* (1981) and urinary unchanged salicylic, salicyluric and gentisic acid were analysed using the procedure published by Waters Associates (1977).

Four days after the single dose study, and after a single day washout of all NSAIDs, a baseline clinical assessment was made and a blood sample was taken. A 42 day chronic study was then commenced with eight of the nine subjects starting on an aspirin dose of 3,900 mg day (3 × 650 mg enteric coated aspirin tablets, twice daily). The remaining subject, MD, who suffered mild Meniere's disease was started on 2,600 mg day^{-1} (2 × 650 mg enteric coated aspirin, twice daily).

Venous blood (5 ml) was collected at 6, 24, 48, and 72 h after the first dose then on days 10, 21, 28 and 35 for the measurement of unbound and total salicylate and salicylurate concentrations (sample taken 3 to 4 h after morning dose). The subjects were also asked to collect overnight urine and note the exact times involved (time of last night void to time of first morning void) on days 2, 3, 4, 10, 21, 28 and 35. The amount of salicylate collected in this time was approximated to the amount of salicylate excreted in 24 h by correcting for the urine creatinine concentration.

On day 41, the subjects took their penultimate dose at 22.00 h and collected all urine subsequent to that dose for the next 12 h. A blood sample and last void of urine was collected at 10.00 h the following morning. The final dose of the chronic study was then taken and blood samples and cumulative urine samples collected at approximately 5, 10, 24, 29 and 34 h after this final dose.

A full serum chemistry profile was also obtained on days 1 and 42 and ESR, liver function tests, albumin and creatinine concentrations were determined on days 1, 10, 21, 28, 35 and 42. Urine volume, pH and creatinine levels were determined accurately and each urine sample was screened for any gross abnormalities using Multistix® (Ames, Australia).

Assessment of response

Subjects were asked to keep a daily diary of their condition for 1 week prior to and throughout the study. This involved recording daily, the duration of early morning stiffness, the level of pain each evening on an analogue scale and the level of wellbeing each evening on an analogue scale. These diaries were collected on days 1, 10, 21, 28, 35 and 42 and the average value for each variable was recorded for the preceding time interval.

In addition to self assessment, on days 1, 10, 21, 28, 35 and 42 each subject was assessed clinically by a rheumatologist. This entailed determining the number of painful joints using Ritchie's index (Ritchie *et al.*, 1968) and assessing the subject's general wellbeing on an analogue scale. Each subject was also questioned about any adverse effects and their perceptions of the medication's efficacy.

Response was measured as the change from baseline values. In the case of the subject's daily perception of pain and wellbeing this was the average of the values recorded on the 2 washout days, and for duration of morning stiffness the average of the two values recorded on the mornings after each washout day. The baseline observed measures were those recorded on day 1. To aid and assess compliance, tablets sufficient to last until the next clinical assessment were provided in a Dosette® container. The empty Dosette® was exchanged for a full container at each visit.

Pharmacokinetic parameters

The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule method from zero time to the time of last sampling with the appropriate correction for the partial area from the final data point to infinity, based on the slope of the terminal exponential phase (Gibaldi & Perrier, 1982);

$$\mathrm{AUC} = {}_0\int^t C\,\mathrm{d}t + \frac{C_t}{k} \qquad (1)$$

where C_t is the concentration at the final collection time, t, and k is the terminal elimination rate constant estimated by regression analysis.

Owing to the nonlinearity of salicylate pharmacokinetics, the plasma half-life of salicylate was approximated as the time taken for

the plasma concentration to fall to 50% of its maximal concentration (Graham *et al.*, 1977). The apparent volume of distribution (V) of salicylate was determined using equation 2 (Graham *et al.*, 1977);

$$V = \frac{\text{AUC}-\text{AUC}_t}{\text{AUC}} \times \frac{D}{C_t} \quad (2)$$

where C_t is concentration of salicylate ($\mu g\ ml^{-1}$) in the plasma at t hours after the dose, D is the equivalent salicylate dose in μg, AUC_t is the area under the curve to time t.

Since the plasma protein binding of salicylate is nonlinear, an average unbound fraction (fu_{av}) for each patient was calculated from the relationship.

$$\text{AUCu(SA)} = fu_{av} \cdot \text{AUC(SA)} \quad (3)$$

where AUCu(SA) and AUC(SA) are the areas under the plasma drug concentration-time curves for unbound and total salicylic acid, respectively.

The Michaelis Menten constants, K_m and V_{max} for the conversion of salicylic acid to salicylurate were estimated by two methods (Roberts *et al.*, 1983). The first method used equation 4 and the nonlinear regression program FUNFIT (Pederson, 1977);

$$C(\text{SU}) = \frac{V_{max} \times C(\text{SA})}{K_m + C(\text{SA})} \times \frac{1}{\text{CL(SU)}} \quad (4)$$

where C(SA) and C(SU) are the plasma concentrations of salicylic acid and salicyluric acid observed after salicylate formation or absorption has ceased, (i.e. after peak plasma salicylate concentration). CL(SU) is the total body clearance of salicylurate which is approximated to renal clearance, since salicylurate is rapidly excreted from the kidneys with only a small fraction undergoing further biotransformation (Levy *et al.*, 1969a). CL(SU) was calculated using the relationship described by Bochner *et al.* (1981);

$$CL(\text{SU}) = \frac{Ae(\text{SU})\ (0_1\ t_1)}{\text{AUC(SU)}\ (0_1\ t_1)} \quad (5)$$

where Ae(SU) (0_1 t_1) is the cumulative amount of SU recovered in the urine during time (0–t_1) and AUC(SU) (0_1 t_1) is the area under the salicylurate plasma concentration-time curve for the same time period.

When there was insufficient decline in plasma salicylurate concentration during the blood sample collection time for the application of a significant regression equation, V_{max} was also estimated using equation 6. This relationship is based on the assumption that at steady-state the rate of formation equals the rate of elimination;

$$V_{max} = \text{CL(SU)} \cdot C(\text{SU})_{ss} \quad (6)$$

where $C(\text{SU})_{ss}$ is the apparent mean steady-state plasma concentration of salicyluric acid.

The whole body clearance of salicylic acid CL(SA) in the single dose study was calculated using equation 7 (Graham *et al.*, 1977);

$$\text{CL(SA)} = \frac{D}{\text{AUC(SA)}} \quad (7)$$

where D is the equivalent salicylate dose.

Parametric statistics (mean and standard deviation (s.d.) or standard error (s.e. mean), t-test, Pearson's correlation coefficient, etc.) were used to analyse the pharmacokinetic data.

Results

Details of the subjects and their drug therapy are provided in Table 1. All subjects had normal albumin ($42 \pm 2\ g\ l^{-1}$), liver function tests (alanine amino transaminase, ALT = 17 ± 3 u l^{-1};

Table 1 Details of subjects

Subject	*Sex*	*Age (years)*	*Weight (kg)*	*Recent* NSAID *therapy*	*Other* RA† *drugs*	*Other drugs*
JB	F	49	82	ASA* diclofenac	hydroxychloroquine	none
BB	F	59	61	ASA* ibuprofen	none	oxprenolol
MD	F	69	51	none	prednisolone, gold	none
KF	M	58	74	ASA* indomethacin	none	none
TL	F	50	62	ASA* indomethacin	gold	none
JM	M	63	80	sulindac, diflusinal	D-penicillamine	none
WM	M	63	72	naproxen	prednisolone, gold	none
HP	F	50	49	naproxen	prednisolone, gold	none
BS	F	49	57	naproxen	gold	none

* ASA therapy ceased 2 weeks prior to study
† At least 6 months duration

Table 2 Summary of plasma salicylate data from the single dose (900 mg aspirin) study.

Subject	C_{max} ($\mu g\ l^{-1}$)	t_{max} (*min*)	*V/BW* ($l\ kg^{-1}$)	$t_{½}$ (*h*)	*CL(SA)* ($ml\ min^{-1}$)	*AUC(SA)* ($mg\ l^{-1}\ h$)	fu_{av}(SA)
JB	48.2	79	0.19	4.2	52.8	217.6	0.097
BB	63.6	70	0.16	7.0	21.8	547.1	0.082
MD	90.4	60	0.13	5.7	19.2	599.7	0,096
KF	65.2	75	0.14	5.9	23.6	487.1	0.090
TL	73.2	56	0.15	3.9	32.6	352.8	0.105
JM	49.3	70	0.18	6.6	26.5	434.4	0.073
WM	58.6	59	0.14	7.2	23.9	480.5	0.090
HP	88.4	74	0.15	4.6	26.8	429.0	0.085
BS	78.3	78	0.15	7.0	22.4	513.2	0.100
Mean	68.4	69	0.15	5.8	27.7	451.3	0.091
± s.d.	15.4	8	0.02	1.3	10.1	113.1	0.010

glutamic alanine transaminase, GT = 8 ± 7 u l^{-1}) and serum biochemistry. Their arthritis was relatively well controlled on their current therapy (erythrocyte sedimentation rate = 18 ± 12 mm in 1 h).

Table 2 summarises the individual pharmacokinetic parameters for salicylate disposition determined in the single dose study, including peak concentration (C_{max}), the time to peak (t_{max}), the apparent volume of distribution corrected for body weight (*V*/BW), plasma half-life ($t_{½}$), total salicylate clearance (CL(SA), total AUC(SA) and the average unbound fraction fu_{av}(SA). The apparent volumes of distribution for salicylate ranged from 6.8 to 15.3 l (mean ± s.d. = 10.1 ± 2.89 l) and were significantly related to body weight (Pearson's correlation coefficient, $r = 0.92$).

Both total and unbound peak plasma salicylate concentrations were significantly correlated with the salicylate dose corrected for body weight, with Pearson's correlation coefficients (r) of 0.96 and 0.93, respectively. There were significant relationships between salicylate clearance (CL(SA) and the total and unbound plasma salicylate concentrations 10 h after the dose, $C(10)$, (Pearson's correlation coefficient, r = 0.69).

There was a significant difference ($P < 0.05$) in the plasma clearance of salicylate between male (0.33 ± 0.07 ml min^{-1} kg^{-1}) and female subjects (0.47 ± 0.11 ml min^{-1} kg^{-1}) when corrected for body weight as in the study of Miners *et al.* (1986). However neither a history of recent salicylate therapy nor of concurrent corticosteroid therapy significantly affected salicylate clearance in the sample.

Table 3 summarises some of the salicylurate pharmacokinetic parameters observed, including the plateau salicylurate concentration ($C(SU)_{ss}$), total salicylurate clearance (CL(SU) and the total unbound Michaelis Menten constants V_{max} and K_m for salicylurate formation.

Two of the nine patients withdrew from the

Table 3 Summary of the plasma salicylate data from the single dose study.

Subject	$C_{ss}(SU)$ ($\mu g\ ml^{-1}$)	*CL(SU)* ($ml\ min^{-1}$)	V_{max} ($mg\ h^{-1}$) *Unbound*	*Total*	K_m** ($mg\ l^{-1}$) *Unbound*	*Total*
JB	2.4	308	38.2	46.2	0.5 ± 0.1	6.9 ± 0.9
BB	1.9	247	39.8	28.1	3.8 ± 1.3	7.6 ± 3.3
MD	2.6	212	24.2	27.0	ILL-DEFINED	23.0 ± 5.4
KF	3.4	160	23.3	29.8	1.9 ± 0.5	18.2 ± 3.3
TL	2.1	197	26.3	24.8	3.8 ± 3.7	10.5 ± 3.6
JM	1.8	310	56.3	33.5	ILL-DEFINED	17.7 ± 5.4
WM	2.4	277	47.5	40.5	ILL-DEFINED	22.1 ± 6.4
HP	2.3	330	64.2	46.0	0.5 ± 0.1	11.3 ± 3.1
BS	2.8	316	58.8	53.2	ILL-DEFINED	9.8 ± 1.5
Mean	2.4	262	42.1	36.6	2.1	14.1
(± s.d.	0.5	61	15.6	10.2	1.7	6.2

* V_m for salicylurate formation determined using Equation 4... $V_{max} = CL(SU) \cdot C_{ss}(SU)$.
** regression estimate for K_m for salicylurate formation determined using Equation 5... $CL(SU) \cdot C_{ss}(SU) = V_{max} \cdot C(SA)/K_m + C(SA)$.

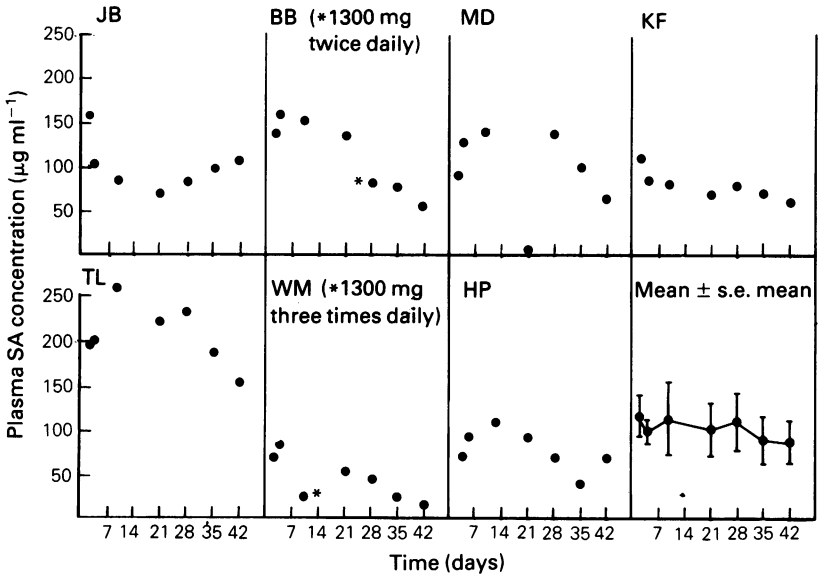

Figure 1 Individual plasma total salicylate (SA) concentrations (3–4 h after the morning dose) over the 6 weeks for the seven patients who completed the study and the mean (± s.e. mean) plasma total SA concentration for patients JB, KF, TL, WM and HP.

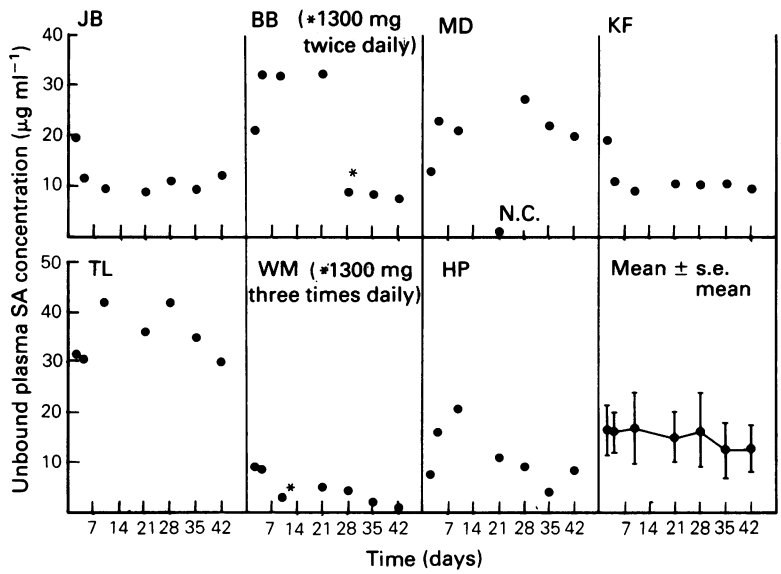

Figure 2 Individual plasma unbound salicylate (SA) concentrations (3–4 h after the morning dose) over the 6 weeks for the seven patients who completed the study and the mean (± s.e. mean) plasma unbound SA concentration for patients JB, KF, TL, WM and HP.

chronic study; JM after only 2 days owing to gastrointestinal intolerance and BS on day 21 owing to ineffectiveness and tinnitus. Figures 1 and 2 show individual total and unbound salicylate concentrations respectively (3 to 4 h after the morning dose), for the seven remaining subjects over the 6 weeks of the study. Because of toxicity it was necessary to reduce BB's dose from 3900 mg to 2600 day^{-1}, and one patient, MD was initially noncompliant. The mean (± s.d.) SA concentration, total and unbound for the five subjects who were compliant and whose total daily dose (3900 mg day^{-1}) was not altered, are also shown in Figures 1 and 2, respectively. Statistical analysis of these data by analysis of variance (ANOVA) indicated that there was a significant decline in the total salicylate concentrations of these five subjects over the 6 weeks ($P < 0.05$). ANOVA of the decline in the unbound drug concentration did not reach statistical significance.

There was, however, a significant decrease in both unbound and total steady-state plasma salicylate concentrations from maximum levels, to the levels on day 42 (*t*-test for correlated sampes, $P < 0.01$, $n = 6$). Total concentrations declined from 145 ± 65 μg ml^{-1} to 91 ± 60 μg ml^{-1}, a mean fall of 42%. Unbound concentrations declined from 22.2 ± 12.0 μg ml^{-1} to 11.8 ± 9.8 μg ml^{-1}, a mean fall of 53%. Only half of the subjects achieved a total SA concentration close to or greater than 150 μg ml^{-1} on the initial dose prescribed, and only one subject, TL had a total plasma drug concentration above 150 μg ml^{-1} on day 42.

The decrease in salicylate concentrations was associated with a corresponding increase in plasma salicylurate concentrations (ANOVA, $P < 0.01$). While this increase was in most cases maximal by day 10, the salicylurate concentration of one subject (KF) appeared to increase after this time. The individual unbound and total salicylurate concentrations over the 6 weeks are plotted and compared with the single dose salicylurate steady-state concentration in Figure 3. This figure also summarises the SU data with a plot of the mean (± s.e. mean) total salicylurate concentrations over this period. There was considerable variation in the mean unbound salicylurate concentrations and no overall trend was observed.

Table 4 compares the average V_{max}, K_m and SU clearance (CL(SU) measured during the single dose study with their respective values on day 42, for the seven subjects who completed the chronic study. The V_{max} for the formation of salicylurate formation appeared to increase significantly during the 42 days (*t*-test, $n = 7$, $P < 0.01$). The SU clearance and K_m were not significantly altered from the values estimated in the single dose study. Despite the significant increase in the V_{max} observed during the study, there was no apparent change in the total salicylate elimination as indicated by the elimination

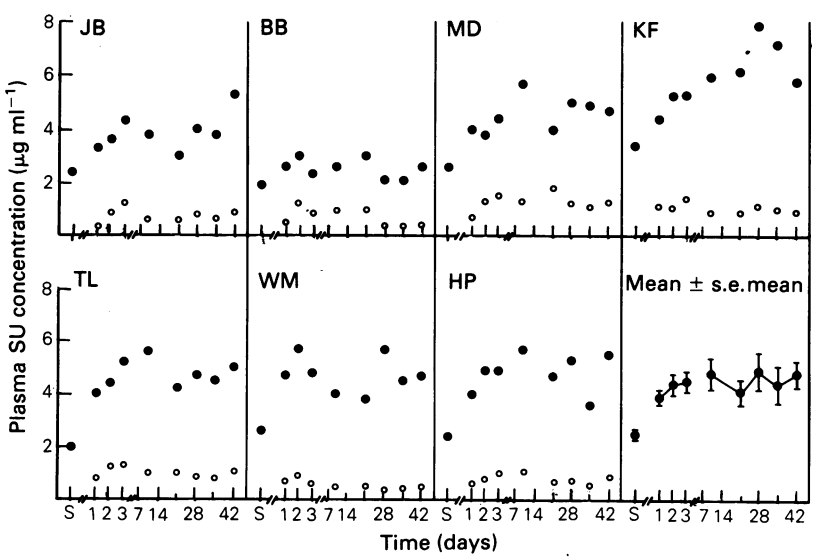

Figure 3 Individual plasma total (•) and unbound (○) salicylurate (SU) concentrations (3–4 h after the morning dose) over the 6 weeks for the seven patients who completed the study and the mean (± s.e. mean) plasma total SA concentrations; the initial data point being the steady-state concentration SU concentration observed in the single-dose study.

Table 4 A comparison of the average Michaelis Menten constants, V_{max}* and K_m, for salicyluric acid formation and salicyluric acid clearance data CL(SU) from the single dose and chronic aspirin studies, for the seven subjects who completed both phases of the study (mean ± s.d. results).

	Single dose study	*Chronic 6 weeks*
Total V_{max} (mg h^{-1})	34.6 ± 9.3	68.3 ± 20.5 †
Unbound V_{max} (mg h^{-1})	37.6 ± 14.8	67.6 ± 20.8 †
Total K_m (mg l^{-1})	14.2 ± 6.8	12.1 ± 7.3
Unbound K_m (mg l^{-1})	2.1 ± 1.7	3.9 ± 2.4
Total CL(SU) (ml min^{-1})	247.3 ± 61.6	242.2 ± 88.4
Unbound CLu(SU) (ml min^{-1})	73.7 ± 37.6	83.5 ± 46.5

* V_{max} estimated using the relationship $V_{max} = CL(SU) \cdot C_{ss}(SU)$
† $P < 0.01$ (t-test, $n = 7$)

Table 5 The calculated salicylate elimination rate constants for the four subjects with sufficient data at the end of the 6 weeks chronic study.

Subject	*Salicylate elimination rate constant* k (h^{-1}) *Single dose*	*Chronic dose*
HP	0.47	0.51
KF	0.16	0.17
BB	0.12	0.09
JB	0.20	0.17
Mean	0.24	0.24
± s.d.	0.16	0.19

rate constants determined from the terminal slopes of the semilog SA concentration-time plots in the single day study and at the end of the 6 week chronic study in those subjects with sufficient data. It was only possible to determine the rate constants for four of the seven subjects who completed the chronic phase of the study. There was not sufficient decline in salicylate concentration during the final sampling for the remaining three subjects (Table 5).

As shown in Table 6 the percentage of the ingested drug excreted as salicylurate, unchanged salicylate and gentisic acid was not significantly altered over the 6 weeks. There was however some variation between subjects in the total SA recovered due in part to the different patterns of absorption. Three subjects, WM, HP and BS reported passing whole enteric coated aspirin tablets in their stool at various times during the chronic study. This helps to explain WM's low average urinary recovery of salicylate throughout the chronic phase and his low average urinary recovery of salicylate of only 47% of the oral dose of salicylate.

During the 6 weeks of the chronic study, the plasma protein binding of salicylurate did not change significantly from that observed in the single dose study, although greater variability in

Table 6 The estimated average 24 h urinary recovery data (mean ± s.d.) during the chronic study for the five subjects receiving 3,900 mg ASA (2,990 mg SA)/day throughout: total salicylate recovered, urinary pH and the percentage excreted as salicyluric acid (SU), unchanged salicylate (SA), and gentisic acid (GA)

Day	*Total urine SA (g)*	*Urine pH*	*% SU*	*% SA(unchanged)*	*% GA*
10	1.70 ± 1.16	6.0 ± 0.5	57.2 ± 6.1	5.5 ± 3.4	2.8 ± 2.3
21	2.74 ± 1.01	6.1 ± 0.5	61.9 ± 12.2	4.0 ± 1.6	2.4 ± 2.0
28	2.62 ± 0.81	5.7 ± 0.4	48.9 ± 6.3	5.0 ± 3.0	2.5 ± 1.6
35	2.33 ± 0.89	6.0 ± 0.6	58.6 ± 5.9	2.9 ± 2.0	2.6 ± 2.0
42	2.35 ± 0.80	5.7 ± 0.5	55.0 ± 8.8	2.9 ± 2.1	2.3 ± 1.1

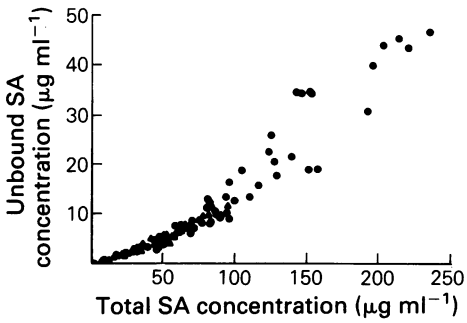

Figure 4 The relationship between unbound salicylate (SA) plasma concentration and total SA concentration observed in both the single dose (▲) and the 6-week chronic aspirin dose (●) studies.

binding was apparent at the higher plasma SA concentrations achieved in the chronic study (Figure 4).

The biochemical parameters, such as albumin and creatinine, were unchanged during the study and the subjects' arthritis remained stable over the 6 weeks, with haemoglobin and ESR levels being essentially contant throughout. Results of the liver function tests, with one exception were also unchanged. Subject BB experienced an elevation in liver transaminase levels, from the baseline values of 15 and 3 u l^{-1} to 73 and 9 u l^{-1} for ALT and GT, respectively, suggesting mild hepatocellular salicylate toxicity.

Clinical response to chronic aspirin therapy was extremely varied with no consistent trends being observed after the initial positive response. Five of the seven patients who completed the 6-week study show improvement from baseline in all measures of response, and two patients (TL, WM) had partial improvement. Patient BS who was forced to withdraw due to a general deterioration of her arthritis experienced a worsening of all parameters of response except the Ritchie (1968) index, which showed improvement.

The incidence of toxicity was extremely high, with five of the nine subjects who entered into the study experiencing an adverse drug event. Subjects BB, MD, LT and BS all experienced tinnitus at some time during the 6 weeks and one subject, JM, experienced gastrointestinal intolerance.

There was a significant difference in both the total and unbound salicylate concentrations between those who experienced and those who did not experience tinnitus (*t*-test, $n = 4$, $P < 0.05$). Subjects who experienced tinnitius had on average higher total and unbound salicylate concentration (203 ± 65 μg ml^{-1} and 47.8 ± 20.5 μg ml^{-1} respectively) compared to those with no tinnitus (116 ± 34 μg ml^{-1} and 16.7 ± 5.7 μg ml^{-1} respectively). In addition to tinnitus, patient BB experienced some mild liver toxicity. Although the tinnitus improved when BB's aspirin dose was reduced, her liver functions tests remained elevated throughout the rest of the study.

Discussion

Determination of pharmacokinetic parameters in patients who have a painful and disabling disease such as rheumatoid arthritis is extremely difficult. Many of the previous single dose studies of salicylate pharmacokinetics in healthy volunteers used a dose equivalent of greater than 1,200 mg of aspirin (Bochner *et al.*, 1981; Day *et al.*, 1988; Netter *et al.*, 1984; Roberts *et al.*, 1983). These studies required at least a 12 h period of frequent blood sampling and more than 24 h of urine collection. This was considered too demanding for most patients with active rheumatoid arthritis and a lower dose of 900 mg was used in this study. This dose was sufficient to saturate the major elimination pathway, the formation of salicyluric acid (Levy, 1965) and previous data suggested that only a 10 h post-dose period of frequent plasma sampling would be required. A preliminary study of a 900 mg aspirin dose in two healthy volunteers indicated that 97% of the dose was recoverable in the urine in 24 h. However, there were significant plasma salicylate and salicylurate concentrations remaining at 10 h after the dose in the patients and thus another plasma sample 14–16 h post-dose may have enabled a more precise pharmacokinetic evaluation.

When estimating pharmacokinetic parameters for salicylate disposition, the complexity of both its distribution and its metabolism necessitate the use of various assumptions and approximations. For instance the calculation of the volume of distribution by the area method used in this study, assumes that the elimination of salicylate is entirely first-order. Also, the estimates of the Michaelis-Menten constants are subject to an error of up to 10% due to the steady-state approximation used in the derivation of the equations (Bochner *et al.*, 1981). However Graham *et al.* (1977) have argued that pharmacokinetic parameters estimated by these methods remain reasonable indicators of intersubject differences and of changes with time of salicylate disposition in the body.

The salicylate concentration-time profiles and other pharmacokinetic data obtained in the

study were similar to that found in other single dose aspirin studies (900 mg to 1,500 mg) with respect to AUC(SA), AUCu(SA), the apparent volume of distribution (V), peak SA plasma concentration (C_{max}), time to peak (t_{max}) and SA clearance (CL(SA) etc. (Cham *et al.*, 1980; Graham *et al.*, 1977; Günsberg *et al.*, 1984; Roberts *et al.*, 1983). Minor differences between the data sets can be attributed to variation in the mg kg^{-1} dose, the age, sex and/or health status of the subjects involved in the various studies.

The Michaelis Menten constants, bound and unbound for salicylurate formation calculated in the current study also appear to be consistent with data from other studies which used plasma data for their estimates (Bochner *et al.*, 1981; Günsberg *et al.*, 1984; Ho *et al.*, 1985; Roberts *et al.*, 1983). There was some variation between the V_{max} values estimated by the two equations used, however these difference were not significant statistically. Because of the decreased precision of the assay at very low salicylate concentrations, unbound regression estimates of V_{max} and K_m were subject to considerable error and could not always be reliably estimated.

Previous studies have shown that salicylate pharmacokinetic parameters may be influenced by the subjects' age and sex (Graham *et al.*, 1977; Ho *et al.*, 1985; Miners *et al.*, 1986; Netter *et al.*, 1985; Roberts *et al.*, 1983). Patient age was not an important consideration in the current study as the subjects involved were of a similar age. Sex differences in salicylate clearance related to hormonal factors (Miners *et al.*, 1986) were, however, apparent. The relationship between oral corticosteroid and SA clearance described by Graham and associates (1977) was not evident, possibly because of the diversity of this small sample.

Graham *et al.* (1977) reported a significant but poor correlation between the apparent volume of distribution for salicylate and patient weight (Pearson's $r = 0.51$). In contrast, these variables were well correlated in the current study ($r = 0.92$). The sample studied by Graham and associates ranged in age from 13 to 66 years and was a mixture of healthy controls, rheumatoid and orthopaedic patients.

The range of maximum concentrations of salicylurate in this single-dose study was in agreement with those reported by Bochner *et al.* (1981) and Ho *et al.* (1985). The average value for SU renal clearance in the current study (mean = 262 ml min^{-1}) was lower than that described in studies of healthy volunteers, which range from 357 ml min^{-1} (Bochner *et al.*, 1981) to 520 ml min^{-1} (Levy, 1965). Günsberg and coworkers (1984) found much greater variability in SU clearance among patients with rheumatoid arthritis, reporting values as low as 124 ml min for SU clearance. Ho *et al.* (1985) suggested that SU clearance was reduced in the elderly, reporting SU clearance values as low as 103 ml min^{-1} for elderly females. Since salicyluric acid is principally eliminated from the body by renal excretion (Levy *et al.*, 1969b) and patients with RA may have impaired renal function due to age, the disease or its treatment, it is understandable that there are differences in the CL(SU) between RA and healthy subjects.

Estimated values for total body clearance of salicylate, CL(SA), which is less directly dependent on renal function, were comparable with values for both healthy and RA subjects described in a previous study (Graham *et al.*, 1977).

There was only a weak correlation between pharmacokinetic parameters estimated in the single dose study and the steady-state salicylate concentrations attained in the chronic study. Graham *et al.* (1977) suggested that salicylate concentrations 12 h after a single dose $C(12)$ were predictive of steady-state concentrations (C_{ss}) during chronic dosing. Because of the lower dose used in the current single dose study, the salicylate concentration at 10 h after the dose $C(10)$ was used in a similar comparison with C_{ss} from the chronic study, with only a weak relationship being evident (Pearson's r, for $C(10)$ and $C_{ss} = 0.46$). Although it could be argued that $C(10)$ was not equivalent to $C(12)$ the correlation observed between CL(SA) and $C(10)$ in the current single dose study was similar to that between CL(SA) and $C(12)$ ($r = 0.69$) reported by Roberts *et al.* (1983) in a 1,200 mg single dose study. Our subjects' erratic absorption of salicylate from the enteric coated dosage form used in the current chronic study, may have been responsible for the poor correlation between $C(10)$ and steady-state salicylate SA concentrations.

The range of maximum salicylate steady-state concentrations (77–266 μg ml^{-1}) was wider than that observed in chronic dosing studies among healthy subjects (daily dose = 3,500–4,500 mg day^{-1}; Rumble *et al.*, 1980 & Olsson, 1983) but was similar to that observed in comparable studies of RA patients (Graham *et al.*, 1977 & Günsberg *et al.*, 1984). Only three subjects, JB, BB and TL, attained the concentration (150 μg ml^{-1}) considered to be associated with significant anti-inflammatory effects (Koch-Weser, 1972). Unfortunately, there is no reference in the literature to an unbound salicylate concentration that is considered therapeutic and it may be that some of the unbound concentrations were

of an order sufficient for anti-inflammatory effectiveness.

Over the 6 weeks of the study there was a significant decrease in both total (18–78%) and unbound salicylate concentrations (22–83%) from the maximum steady-state values. There was large intersubject variation in both the magnitude and the time-course of the decline and some patients had additional problems such as toxicity, initial noncompliance and erratic absorption. Notwithstanding these problems, there was an overall steady decline in C_{ss} for total and unbound salicylate. Rumble *et al.* (1980) and Olsson (1983) have previously reported similar findings during chronic aspirin studies of 5 to 6 weeks among healthy volunteers, observing an 8–57% and a 50–65% decrease in total salicylate concentrations, respectively. Although there are no comparable data on unbound salicylate in the literature, Rumble and associates (1980) also reported a decline in salivary salicylate concentrations during their study as a reflection of the unbound salicylate concentration.

Urinary recovery data indicated that changes in absorption could not account for all of the decline in salicylate concentrations. Individual subjects reported problems with absorption form the enteric coated dosage form and this is reflected in the variability of their urinary recovery data. In the other subjects, who made no report of absorption problems, the recovery was in agreement with the data of Rumble *et al.* (1980), who reported an average recovery rate of 70% of the daily dose in a 24 h urine specimen. Rumble *et al.* (1980) also reported no significant change in the relative proportions of salicylate and its metabolites, although they observed a nonsignificant increase in the percentage excreted as salicylurate. There was no significant change in the relative proportions recovered as gentisic, salicyluric and salicylic acids in the urine in this study.

The plasma binding of salicylate was unchanged during the six-week study. Salicylurate binding was affected by high plasma SA concentrations, suggesting a common binding site and confirming the observations made by Bochner *et al.* (1981). Data from our single-dose dose study were also consistent with this theory. However, this displacement interaction should not have had a significant influence on the concentration of unbound SA or SA clearance, since the plasma concentrations of SA were much greater than those of SU and the extent of SU binding (75–80%) was less than that for SA.

Several authors have explained the decline in salicylate concentrations by induction of the enzymatic process responsible for the formation of salicylurate (Day *et al.*, 1983, 1988; Furst *et al.*, 1977; Rumble *et al.*, 1980). Consistent with this theory there was a significant increase in both total and unbound V_{max} for salicylurate formation. It was not possible to determine a regression estimate of V_{max} for all subjects, owing to insufficient decline in plasma concentrations. However, when both methods of calculation were possible the estimates were comparable.

Accompanying the increase in V_{max} and the decline in salicylate concentrations during the 6-week study, there was an increase in steady-state plasma salicylurate concentrations. Overall the pattern of increase in SU concentration during the 6 weeks paralleled the descriptions of the change in V_{max} for SU formation from previous studies; i.e. Furst *et al.* (1977) observed an increase in V_{max} for SU formation within 3 days of starting salicylate therapy, Günsberg *et al.* (1984) suggested that the increase in V_{max} was not complete within 7 days of starting therapy and Day *et al.* (1983, 1988) found that induction was maximal at 2 weeks. Our unpublished data also suggest that there is a limit to the increase. The range of SU plasma concentrations (2.5–8.4 $\mu g\ ml^{-1}$) observed in a screening study of 47 subjects taking aspirin chronically for 1 to 10 years, was similar to SU concentrations found during the current six-week study (2.6–7.9 $\mu g\ ml^{-1}$). At the start of the chronic study, there was a rather steep initial increase in the plasma SU concentration from that observed in the single dose study, which was probably related to the dose change.

The renal clearance of salicylurate and its Km remained unchanged during the study, supporting the concept that increases in salicylurate plasma concentrations were most likely due to increases in the rate of SU formation. In spite of this apparent increase in salicylurate formation there was no evidence of a corresponding increase in the overall elimination rate of salicylate from the body; the terminal slopes of salicylate semilog concentration-time plots in the single dose study, being comparable with those at the end of the 6 weeks for those subjects with sufficient data. This does not preclude an increase in V_{max}, since the lack of change in the elimination rate constants can be explained in terms of parallel change in the apparent volume of distribution (Gibaldi & Perrier, 1982). However, the absence of an increase in the percentage excreted as salicylurate during the 6 weeks, suggests that the decline in steady-state salicylate concentrations may not be explained simply by induction of salicylurate formation, but may also

involve other metabolic pathways (*Day et al.*, 1988).

Because of the diversity of the response to aspirin therapy, it was not possible to test the initial hypothesis that the decrease in steady-state plasma salicylate concentrations may affect clinical response to therapy. All patients had an initial positive response to one or all of the parameters of response and in most cases this was maintained.

The high level of toxicity was an unexpected problem. All but one of the adverse reactions which occurred in the current study, are considered concentration-related effects; in some cases they were evident at total SA concentrations below the accepted therapeutic range. Gurwich *et al.* (1984) also reported dose-related adverse effects among RA patients at concentrations lower than those generally considered toxic.

Both the total and unbound salicylate concentrations of those patients who experienced tinnitus were significantly higher than those without adverse effects and, as in the studies of Müller *et al.* (1975) and Olsson (1983), the tinnitus disappeared when the salicylate concentration decreased. The high incidence of toxicity in the study may have been associated with the twice daily dosing schedule. A study by Miller (1978) found that a higer incidence of tinnitus was associated with dosage schedules involving larger doses of aspirin at longer time intervals.

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